

## II. LISTING OF THE CLAIMS

The following listing of the claims replaces all prior versions, listing and amendments to the claims.

1. (Currently Amended) A method for reconstituting IKK in yeast comprising the steps of:
  - a. subcloning an IKK subunit gene into yeast expression vectors;
  - b. transforming said yeast expression vectors into yeast;
  - c. growing said yeast in a selective liquid media; and
  - d. controllably inducing the expression of said ~~IKK subunits~~ subunit by means of ~~inducible promoters~~ a promoter.
2. (Currently Amended) The method of claim 1, further comprising the steps of:
  - a. lysing said yeast;
  - b. extracting said an IKK protein produced by said IKK subunit gene; and
  - c. purifying said IKK protein.
3. (Currently Amended) The method of claim 1, wherein said yeast expression vectors ~~contain a selection~~ further comprise a gene encoding a selectable marker.
4. (Currently Amended) The method of claim 3, wherein said ~~selection~~ selectable marker gene encodes ~~is~~ leucine, histidine, tryptophan, or uracil.
5. (Currently Amended) The method of claim 1, wherein said ~~yeast expression vectors~~ contain IKK subunits further comprise a polynucleotide encoding a tag.
6. (Currently Amended) The method of claim 1, wherein said tag is myc, HA, ~~or~~ FLAG or 6his.
7. (Currently Amended) The method of claim 1, wherein ~~said yeast expression vectors~~ contain an said promoter is an inducible promoter or a constitutive promoter.
8. (Previously Amended) The method of claim 7, wherein said inducible promoter is methionine or galactose.

Any. Dkt: USC 7064

9. (Previously Amended) The method of claim 7, wherein said constitutive promoter is alcohol dehydrogenase.
10. (Original Claim) The method of claim 1, wherein said IKK subunit is  $IKK\alpha$ .
11. (Original Claim) The method of claim 1, wherein said IKK subunit is  $IKK\beta$ .
12. (Original Claim) The method of claim 1, wherein said IKK subunit is  $IKK\gamma$ .
13. (Currently Amended) The method of claim 1, wherein said IKK subunit comprises one or more of a combination of  $IKK\alpha$ ,  $IKK\beta$ , and  $IKK\gamma$ .
14. (Currently Amended) The method of claim 10, 11 or 13 wherein said IKK subunit is  $IKK\alpha$  and  $IKK\beta$  subunits are subcloned into pESC ura or pESC trp vectors wherein a galactose promoter region is replaced with a met promoter from a leu(met) vector.
15. (Currently Amended) The method of claim 12 or 13, wherein said  $IKK\gamma$ -IKK subunit expression is subcloned into said leu(met) vector regulated by said promoter.
16. (Currently Amended) The method of claim 12 or 13, wherein said  $IKK\gamma$ -IKK subunit expression is subcloned into the pES-86(+) a pESC expression vector wherein constitutive and said  $IKK$  expression is induced under the alcohol dehydrogenase by a promoter.
17. (Original) The method of claim 1, wherein said yeast is *Saccharomyces cerevisiae*.
18. (Original) The method of claim 1, wherein said IKK is mammalian IKK.
19. (Previously Amended) The method of claim 18, wherein said mammalian IKK is human IKK.
20. (Original) The method of claim 1, wherein said vectors are plasmids, small yeast chromosomes or cosmids.
21. (Original) The method of claim 1, wherein said selective liquid media is a non-inducing drop-out media.
21. (Currently Amended) The method of claim 1, wherein said purified IKK protein gene encodes wild-type IKK is substantially homologous to IKK isolated from wild-type cells.

Atty. Dkt: USC 7064

22. (Currently Amended) The method of claim 1, wherein said ~~purified~~ IKK gene protein is mutated.
23. (Original) A heterologously expressed IKK complex, wherein said IKK is expressed by yeast.
24. (Withdrawn) The composition of claim 24, wherein said IKK complex is comprised of IKK $\alpha$ , IKK $\beta$ , and IKK $\gamma$  subunits.
25. (Withdrawn) The composition of claim 24, wherein said IKK complex is produced by the method of claim 1.
26. (Withdrawn) A heterologously expressed IKK complex, wherein said IKK $\gamma$  protein subunit regulates phosphorylation of serine residues in the activation of T loop kinase domain of IKK catalytic subunits.
27. (Withdrawn) The method of claim 27, wherein said IKK complex is activated by the dephosphorylation of  $\gamma$ BD serines.
28. (Withdrawn) A yeast cell containing an expressible copy of a gene encoding a subunit of IKK.
29. (Withdrawn and Previously Amended) The yeast cell of claim 29 which is transformed with a yeast expression vector which contains the expressible copy of the gene encoding IKK $\alpha$ , IKK $\beta$ , or IKK $\gamma$ .
30. (Withdrawn and Previously Amended) The yeast cell of claim 29 which is transformed by the method of claim 1.
31. (Withdrawn) A method for identifying upstream regulators of IKK complex, comprising the steps of:
- mutating the genes of one or more said IKK subunits;
  - subcloning genes for IKK subunits into yeast expression vectors;
  - transforming said yeast expression vectors into yeast;

- d. growing said yeast in a selective liquid media;
  - e. controllably inducing the expression of said IKK subunits by means of inducible promoters;
  - f. lysing said yeast;
  - g. extracting said IKK protein;
  - h. purifying said IKK protein; and
  - i. comparing kinase activity of said IKK protein with wild type IKK.
32. (Withdrawn) The method of claim 32, wherein said mutation is on a binding domain.
33. (Withdrawn and Previously Amended) The method of claim 33, wherein said mutation mimics the biochemical characteristics of said binding site when bound.
34. (Withdrawn and Previously Amended) The method of claim 33, wherein said mutation prevents binding at said domain site.
35. (Withdrawn) The method of claim 32, wherein said mutation changes serines to alanines.
36. (Withdrawn) The method of claim 32, wherein said mutation changes serines to glutamic acid.
37. (Withdrawn) A method for assaying IKK activity in situ in yeast comprising the steps of:
- a. subcloning genes for IKK subunits into first yeast expression vectors;
  - b. transforming said first yeast expression vectors into yeast;
  - c. subcloning HeLa cell cDNA into second yeast expression vectors;
  - d. transforming said yeast expression vectors into said yeast;
  - e. replica plating said yeast;
  - f. growing said yeast on membranes on selective non-inducing medium
  - g. inducing said yeast to produce IKK protein;
  - h. fixing said IKK protein;

Any. Dkt: USC 7064

39. (Withdrawn and Previously Amended) The method of claim 38, further comprising the step of sequencing said positive clones.

40. (Withdrawn and Previously Amended) The method of claim 38, further comprising the steps of:

- a. transforming said positive clone into yeast;
- b. growing said yeast in a selective liquid media;
- c. controllably inducing the expression of said clones by means of inducible promoters.

41. (Withdrawn and Previously Amended) The method of claim 0, further comprising the steps of:

- a. transforming said positive clone into yeast;
- b. growing said yeast in a selective liquid media;
- c. controllably inducing the expression of said clones by means of inducible promoters.